5-9-2019

The Effect of Simulated Storm Passage on the Behavior of Whitespotted Bamboo Sharks, Chiloscyllium Plagiosum and Golden Shiners, Notemigonus Chrysoleucas

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The effect of simulated storm passage on the behavior of Whitespotted bamboo sharks, *Chiloscyllium plagiosum* and Golden shiners, *Notemigonus chrysoleucas*

by

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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford

May 2019

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Acknowledgments

ABSTRACT

JORDAN ALYSE HEALY: The effect of simulated storm passage on the behavior of Whitespotted bamboo sharks, *Chiloscyllium plagiosum* and Golden shiners, *Notemigonus chrysoleucas*

(Under the direction of Dr. Glenn Parsons)

Environmental changes during a storm can affect animal behavior; for example, tagged sharks leave shallow waters during tropical storms. While changes in shark behavior during storm passage have been documented, there have been no experimental tests to determine which environmental parameters are responsible. In this study, we experimentally manipulated two environmental cues: barometric pressure, and thunder sounds, to determine their effect on whitespotted bamboo sharks, *Chiloscyllium plagiosum,* and golden shiners, *Notemigonus crysoleucas*. Elasmobranchs do not have a swim bladder to detect pressure change; therefore, we used golden shiners to compare the reaction of a fish with a swim bladder. The pressure was decreased by 1.52 cmHg from a high atmospheric pressure (75.69 cmHg – 76. 35 cmHg) over three hours, and the lowest pressure (1.52 cmHg from the atmospheric pressure) was held for an hour while activity was recorded. There was no difference between the activity during the treatment of manipulated pressure and treatment of constant pressure in *C. plagiosum*. When observing time interval of decreasing pressure (Hours 0 to 3), *N. crysoleucas* was more active when the pressure was decreased than when pressure was not decreased. When observing the activity of the golden shiners, we found that the golden shiners were less active as the pressure continued to decrease. *C. plagiosum* were also exposed to recorded thunder sounds, and a control of no thunder sounds. We documented the activity during each trial. There was no significant

difference in the activity of *C. plagiosum* between the treatment of thunder sounds and the treatment of no thunder sounds. The daily fluctuations of activity may have been a confounding variable to the activity resulting from the treatment. While many of these results are preliminary, the results are an interesting insight on how these two species, white spotted bamboo sharks and golden shiners, possibly react to various isolated cues present in a storm.

TABLE OF CONTENTS

List of Figures

Introduction

Many environmental factors change during a storm including rainfall, wind speed, barometric pressure (Heupel et al., 2003; Udyawer et al., 2013), and presence and strengths of electric fields (Maggio et al., 2005). These environmental changes can cause lead to behavioral changes in animals (Udyawer et al., 2013). Modification of animal behavior in response to environmental changes during storms has been observed in invertebrates (Udyawer et al., 2013), teleosts (Watterson et al.,1998), and elasmobranchs (Heupel et al., 2003).

Heupel et al. (2003) speculated that changes in environmental cues caused by the landfall of a tropical storm caused blacktip sharks, *Carcharhinus limbatus*, to leave their shallow nursery area. Udyawer et al. (2013) tracked shark movements during tropical storms and concluded that the ability to detect and avoid adverse conditions, such as storm surges or large waves, by fleeing to deeper water could provide a competitive advantage. The synchronicity of the departures suggested that this behavior was triggered by an environmental cue associated with the storm. In the study tracking blacktip sharks, rainfall, barometric pressure, and wind speed were correlated with the time period that the blacktips left the nursery area. Heupel et al. (2003) correlated the flight behavior of *C. limbatus* sharks from shallow water and, based on anecdotal evidence, concluded that the drop in barometric pressure was the environmental cue for the *C. limbatus* exodus behavior. Another cue associated with storms that was not considered in the previously mentioned study is the presence of auditory cues such as thunder. The presence of anthropogenic noises elicits a startle response in fish (Peng, Zhao, & Liu, 2015).

We selected two species, whitespotted bamboo sharks, *Chiloscyllium plagiosum* (Orectolobiformes: Hemiscylliidae) and golden shiners, *Notemigonus crysoleucas* (Cypriniformes: Cyprinidae) for this study. Whitespotted bamboo sharks are a benthic species from the Indo-Pacific regions (Chen, Chen, Liu, & Wang, 2007). We used this organism because their size and adaptability make them ideal for aquarium use. Golden shiners were selected for an additional species in this study. *N. crysoleucas* are the most prevalent baitfish across the United States, making them readily available and easy to maintain in aquaria. They are native to eastern North America and can inhabit most types of standing water (Green, 2006).

The objective of our study was to determine if the presence of environmental storm cues would produce a change in activity in *C. plagiosum*. The two environmental cues tested in this study were barometric pressure and thunder. In order to compare different orders of fish, we tested *N. crysoleucas* to the cue of decreased pressure. We wanted to compare the two different proposed mechanisms for detecting changes in pressure; the swim bladder in the golden shiners and the vestibular hair cells in whitespotted bamboo sharks (Fraser & Shelmerdine, 2002; McCutcheon, 1966). Due to the results of the previously mentioned studies, we hypothesized that pressure changes would result in increased activity in both *C. plagiosum* and *N. crysoleucas*. Similarly, we hypothesized that the presence of thunder would result in increased activity in *C. plagiosum*.

Materials and Methods

Effect of Pressure and Activity

In order to manipulate barometric pressure, a barometric pressure chamber was constructed using a fiberglass tank (77.5 cm tall, 122.5 cm wide), a Plexiglass cover (132.08 cm diameter, 0.375 cm thickness), and a vacuum pump. We sealed the tank with Plexiglass, silicone, and C clamps (Figure 1). The vacuum hose had a release valve to manipulate the fall and rise of the barometric pressure. A GoPro Hero 4 recording device was used to film activity during the trials. An electric barometer within the tank detected the change in barometric pressure.

During experimentation, one juvenile whitespotted bamboo shark (Figure 2) was placed in the tank and was acclimated for 12 hours. After the acclimation period, the trial began. Trials were only conducted on days with an environmental pressure above 75.69 cmHg. During the treatment trial, the barometric pressure was reduced by 1.52 cmHg from the starting atmospheric environmental pressure (75.69 cmHg – 76. 35 cmHg) over the course of three hours. The lowest pressure (74.47 cmHg – 74.83 cmHg) was then held constant for an hour. The pressure was slowly raised to atmospheric pressure over the course of three additional hours. During the control trial, the barometric pressure was held constant for seven hours. The vacuum pump was running during the control to ensure that noise from the pump was not a confounding variable. In total, two control trials and two treatment trials were conducted with two individual whitespotted bamboo sharks. There was a minimum two-week span in between using the same individuals.

These treatments were also conducted with golden shiners (Figure 3) for a non-elasmobranch comparison. Twelve golden shiners were placed in the barometric pressure chamber in order to not have a confounding variable of isolation stress with this species. The same decrease in pressure (1.52 cmHg), acclimation period, experiment time, and control treatments were utilized when using the golden shiners.

The seven-hour experimental period was filmed, and the activity was tracked from the film. The activity was measured using the GoPro Hero 4 videos and the squares on a grid drawn on the clear Plexiglas top of the chamber. The dimensions of each square on the grid were 100 cm^2. We calculated total activity during the trials that had manipulated pressure and the trials without consistent pressure for both *C. plagiosum* and *N. crysoleucas.* We determined the total activity by counting the total 100 cm^2 squares moved by the fish. The activity was correlated with the time and the pressure at these times. This allowed us to sum the data for the various intervals such as the intervals of decreasing pressure, low pressure, and increasing pressure. The sum of the activity could be compared if the sum was over the same amount of time. If the time differed within the interval, the activity per second was calculated by dividing the sum of activity over the total amount of seconds within that interval.

Effect of Thunder on Activity

Shark activity was recorded with a GoPro Hero 4 during treatment (recorded thunder) and control (no thunder) trials. During treatments, recorded thunder (EPIC THUNDER & RAIN, https://www.youtube.com/watch?v=nDq6TstdEi8&t=10181s) was played on a speaker approximately 60 cm away from the aquarium for 50 minutes at a constant volume. During control treatments, activity was recorded with the absence of thunder sounds for the same time period. The sharks were exposed to the trials within their tank which contained two sharks at a time. We conducted the experiment within the main aquarium in order to observe their natural response without the additional stress of moving the sharks. We conducted the first trial with the treatment of thunder noises was followed by observing the activity after the cessation of thunder, however, because of concerns that the previous treatment could affect the activity of the subsequent trial, the experiment was redesigned. For our experiment, the trials were conducted on separate days with similar environmental conditions (lack of storms and atmospheric pressure above 75.44 cmHg). The distance (cm) the shark moved on the video was measured relative to the dimensions of the aquarium (Figure 4). When the shark moved, the distance (cm) traveled was denoted based on the length of the tank which was used to approximate the distance the shark moved. The total activity of the fifty-minute trial was measured by calculating the distance traveled. The sum of the measurements was used for statistical analysis. There were four trials conducted with the presence of thunder and four trials conducted with the absence of thunder.

Statistics

A regression was used to examine the continuous relationship between activity per second and the change in pressure during the experiments with *N. crysoleucas*. We also evaluated the time interval in which the pressure was decreased (Hours 0 to 3) in order to compare decreasing pressure to the same time interval within the control. The activity was defined as the number of 100 cm^2 squares on the grid that the fish traveled. In order to conduct a Welch's t-test, a square root transformation of the activity was performed in order to account for a skewed distribution of the variable.

The sum of the activity (distance traveled in cm) in each trial involving thunder noises or the absence of thunder noises was measured in order to compare the activity during the trials with the auditory cue. Welch's two-sample t-test was used for statistical testing.

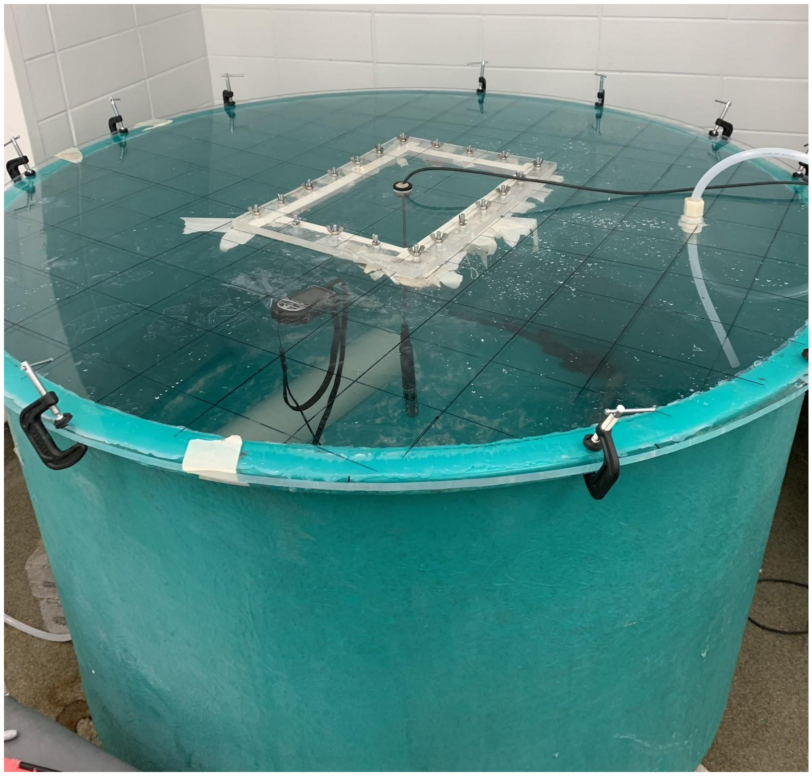

Figure 1 – The pressure chamber used for the experiments testing the effects of pressure on activity on *Chiloscyllium plagiosum* and *Notemigonus crysoleucas*.

Figure 2 – Picture of *Chiloscyllium plagiosum,* which was used for both the experiments: observing activity during pressure change and observing activity during the presence of thunder.

Figure 3 – Image of *Notemigonus crysoleucas,* which was used in experiments observing activity during changes in pressure.

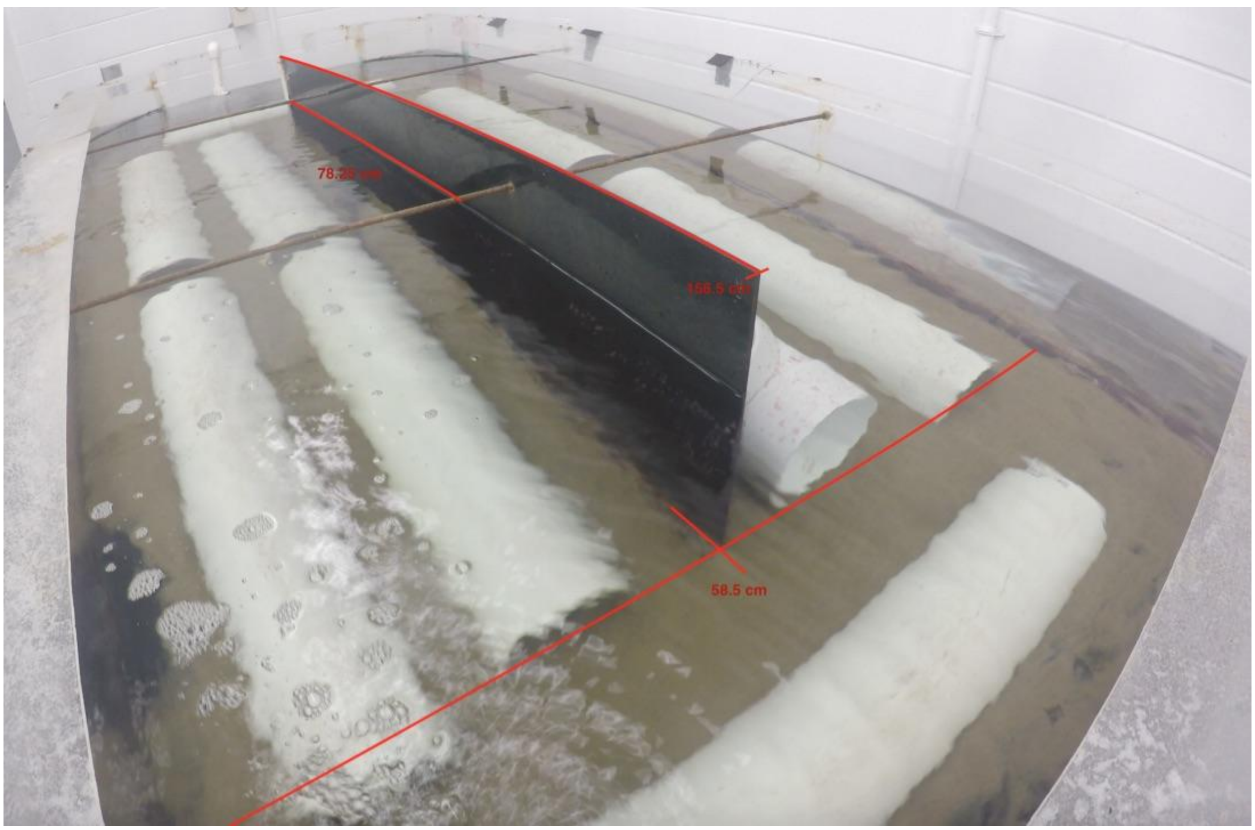

Figure 4 – The holding tank used for experiment testing if the auditory cue of thunder affected activity in *Chiloscyllium plagiosum.*

Results

Pressure and Activity

Pressure had no effect on the activity of whitespotted bamboo sharks. There was no change in activity over the course of any pressure experiment. There was no movement (0) in the trials when pressure was changed and there was no movement (0) when the pressure was not changed.

There was a significant difference between the activity of the golden shiners when pressure was decreased and when pressure was not changed when evaluating the time interval of 0 to 3 hours. ($t(716.86)=-3.83$, $p=0.00014$;Figure 5).

Pressure and activity were plotted over the time of trials when pressure was declined with *N. crysoleucas* (Figure 6). There was a significant relationship between the amount of decrease in pressure (cmHg) and activity per second ($R_2=0.249881194$, $F(1, 24)=7.6618096$, $p=4.44769 \times 10^{-7}$; Figure 7)

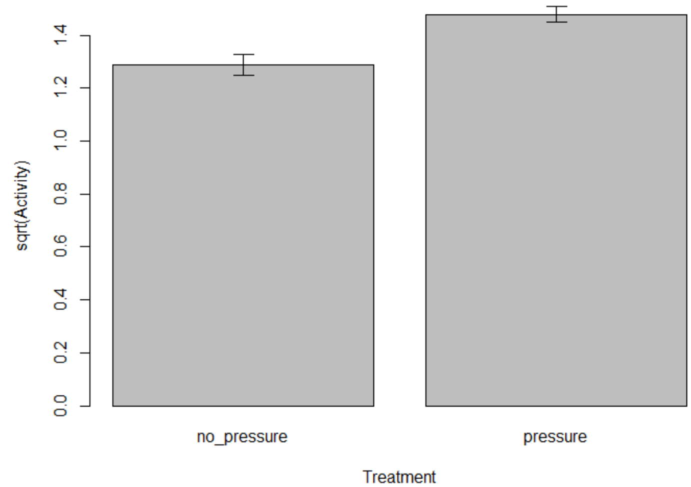

Figure 5 – The difference in the square root of activity (activity is defined as the number of $100cm^2$ squares traveled by the fish) during the first three hours of *N. crysoleucas* between treatments (pressure decrease and no pressure change)

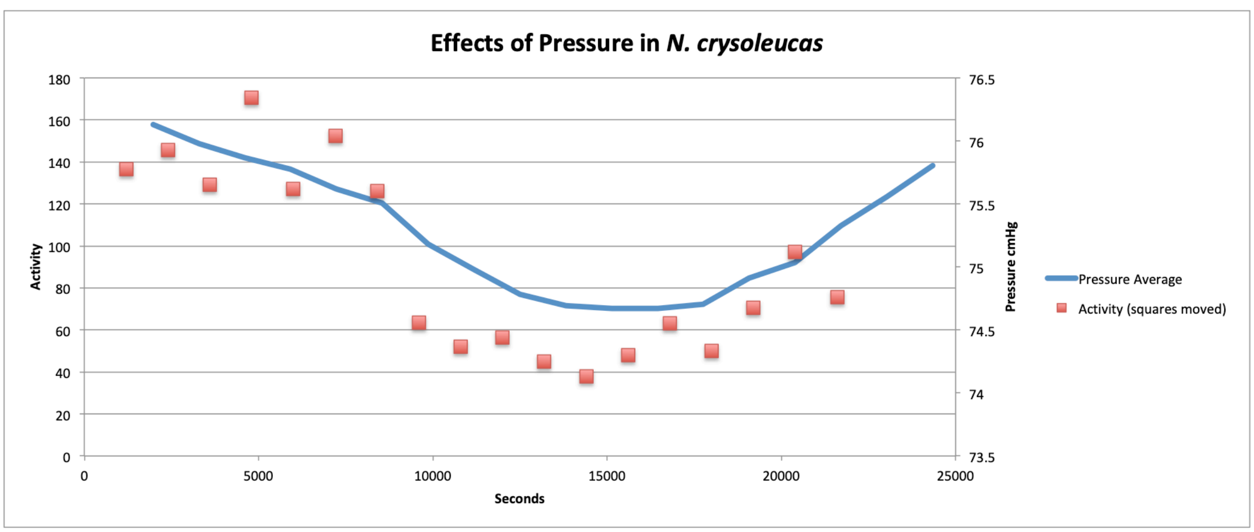

Figure 6 – The blue line represents the barometric pressure (cmHg) and the red dots represent mean activity (activity is defined as the number of $100cm^2$ squares traveled by the fish) for *Notemigonus crysoleucas* plotted over time (seconds)

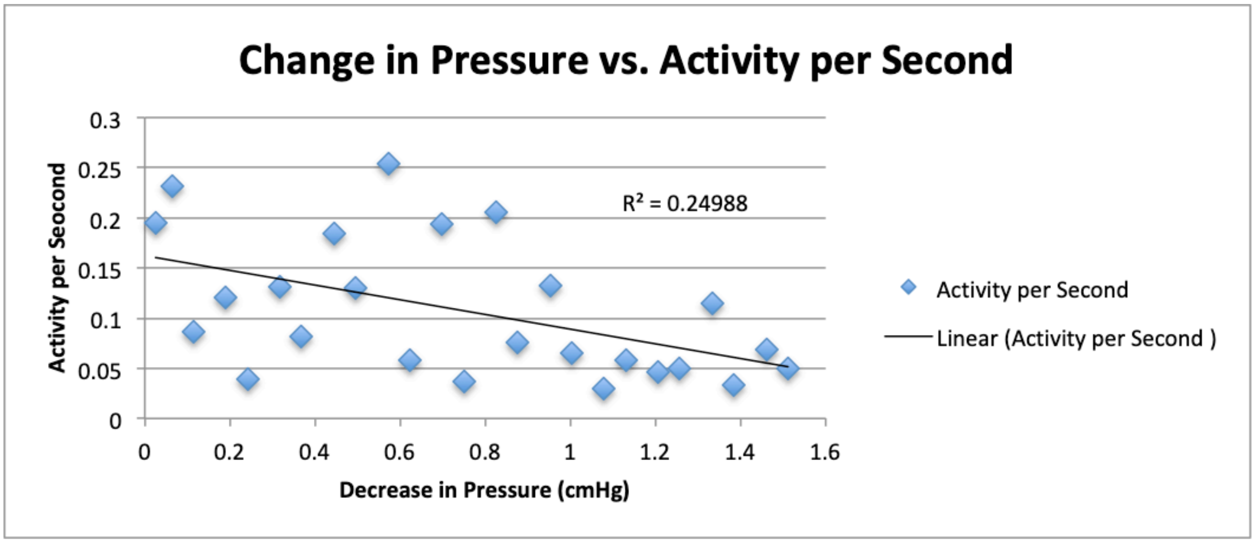

Figure 7 – Regression of activity (activity is defined as the number of $100cm^2$ squares traveled by the fish) per second of *Notemigonus crysoleucas* based on the change in pressure from the starting atmospheric pressure

Thunder and Activity

The effect of thunder on activity was observed over 1.5 hours with sounds of thunder playing followed by approximately 2 hours of no thunder. The results from our first test showed higher activity during a treatment trial (thunder) than control trial (no thunder) conducted consecutively (Figure 8). The distance (cm) traveled per minute was higher in the thunder trials (Figure 9).

When the trials were conducted on separate days, there was no significant difference between the activity during treatment trials of the auditory cue of thunder and the control trials without the auditory cue of thunder ($t(3.22)=1.2374$, $p=0.2986$; Figure 10)

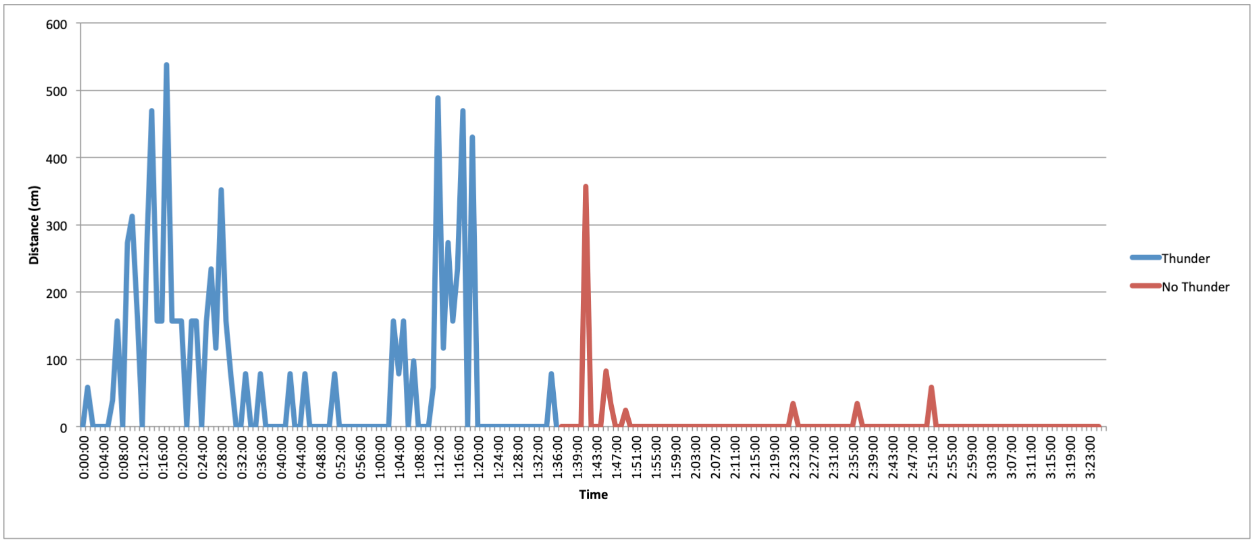

Figure 8 – The effect of thunder on distance (cm) traveled by whitespotted bamboo sharks during the first trial. The sum of the activity each minute during thunder playback (Blue) and activity after cessation of thunder (Red).

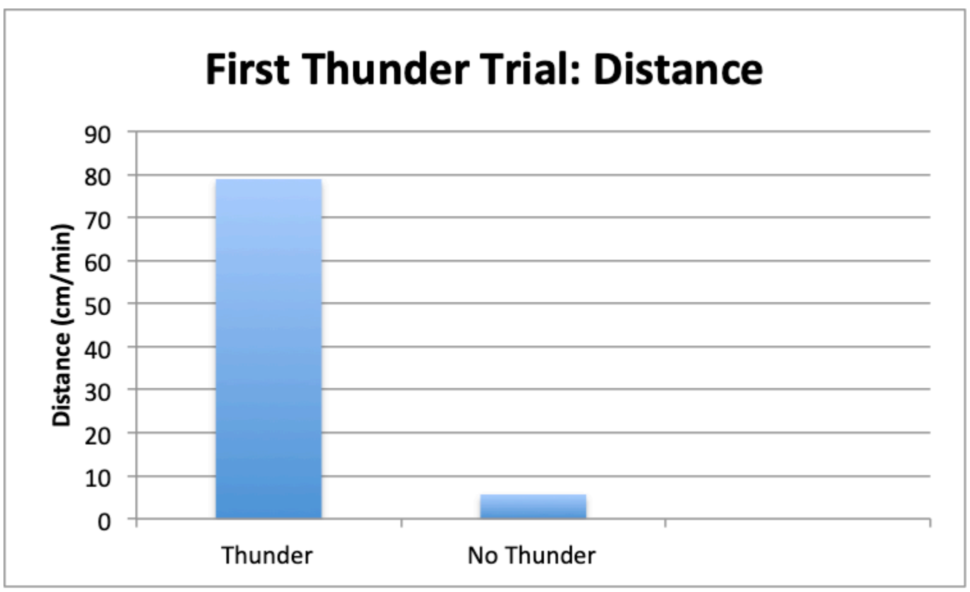

Figure 9 – The effect of thunder on activity in white spotted bamboo sharks. The average distance per minute (cm/min) was determined for the interval when thunder was playing and after the cessation of thunder (no thunder)

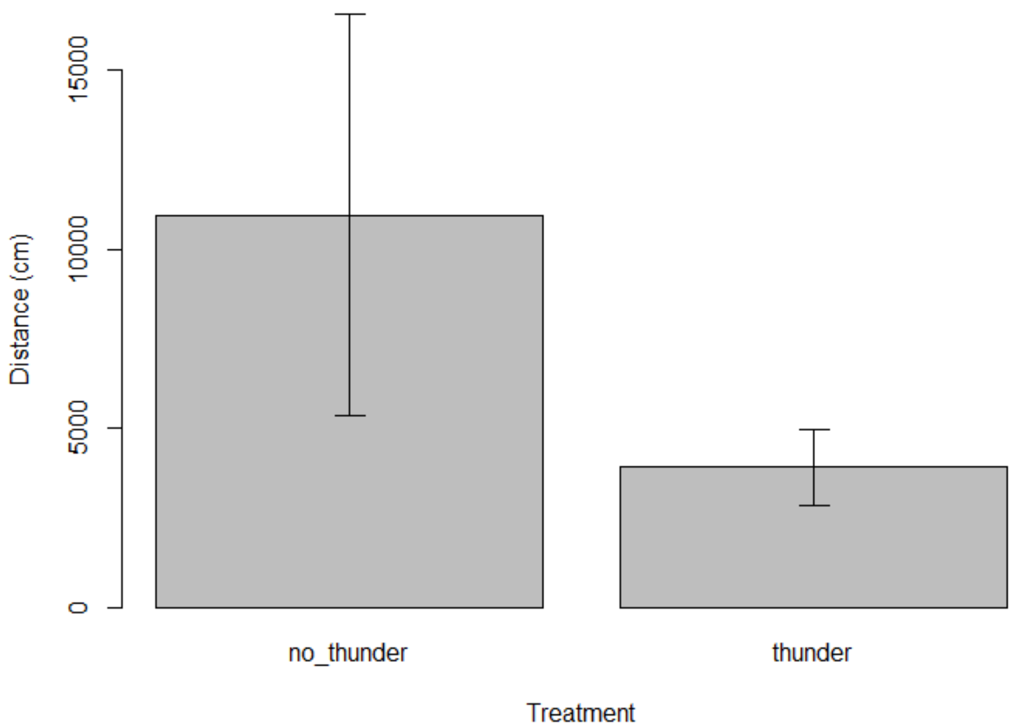

Figure 10 – The effect of thunder on activity in whitespotted bamboo sharks. In these experiments, activity (distance traveled in cm) was monitored while thunder was played for 50 minutes at randomly chosen times of the day. Control experiments were the same except no thunder was played.

Discussion

The many environmental changes associated with changing weather patterns such as storm systems may provide cues by which animals detect an approaching storm. Storms can cause increases in cortisol levels, which results in the development of a stress response (Wingfield, 2005). In natural habitats, some animals have the ability to alter behavior in order to avoid storms, such as *C. limbatus* fleeing to deeper water because of a tropical storm (Heupel et al., 2003). Other studies have examined the relationship between declining barometric pressure and food intake in the white-crowned sparrow, *Zonotrichia leucophrys* (Breuner, Sprague, Patterson, & Woods, 2013). In that study, declining barometric pressure stimulated food intake, suggesting that *Z. leucophrys* can sense and respond to declining pressure. Captive animals that cannot alter their activity as they could in their natural habitat may experience stress. If it is possible to determine which environmental cue stimulates activity change during a storm, animal handlers could possibly make alterations when large storms are approaching.

Sharks are Elasmobranchs, a subclass of cartilaginous fish containing sharks, rays, and skates. Elasmobranchs lack a swim bladder, an organ that other fishes possibly use to detect changes in hydrostatic pressure (Holbrook & de Perera, 2011). Sharks are sensitive to changes in hydrostatic pressure even without a swim bladder because sharks are able to detect subtle changes through their mechanoreceptors in the inner ear (Fraser & Shelmerdine, 2002). Previous studies have found that teleosts were sensitive to rapid negative pressure (e.g., a change of 20 cmHg in 0.5 sec) and displayed an escape reaction (McCutcheon, 1966).

Pressure and Activity

Heupel et al. (2003) presumed that pressure change led to the reaction of *C. limbatus* since Tropical Storm Gabrielle led to a decrease in barometric pressure of 13mb (0.97 cmHg). However, as that study did not empirically test this hypothesis, the behavioral change observed could have been caused by changes in atmospheric charge, increased thunder, rain striking the surface of the ocean, the sound of increased wave activity due to the increase in wind velocity, as well as changes in pressure.

An additional study tracked shark movements during tropical storms and found that *Carcharhinus amboinensis*, *C. sorrah*, *C. tilstoni*, and *C. limbatus* exhibited a flight response as a storm approached (Udyawer et al., 2013). The study noted that different species had different points of fleeing, but within the same species, there was synchronicity between departure times. The majority of the tagged *C. tilstoni* were juvenile and would not have previously experienced a storm, so their flight response to an approaching storm suggests that detection and reaction to storms may be an innate response (Udyawer et al., 2013).

While a barometric pressure effect on activity in sharks has yet to be established, other fishes have been observed to respond to pressure change. McCutcheon (1966) found that teleosts were sensitive to rapid negative pressure (e.g., a change of 20 cmHg in 0.5 sec) and displayed an escape reaction. Fish communication can be negatively impacted by the presence of tropical storms and fish either stopped croaking or left the monitored site (Gottesman et al., 2017).

C. plagiosum did not move during the trials involving both the treatment of manipulated pressure and the treatment without manipulated pressure. Our experiment had a low sample size because there was a limited number of *C. plagiosum* available. While more trials are necessary to determine the effect of pressure, this study provides a potential finding that pressure may not be the environmental cue that causes a change in activity during a storm.

While both *C. plagiosum* and *C. limbatus* are elasmobranchs, *C. limbatus* is a pelagic species, and *C. plagiosum* is a benthic species. A pelagic species of shark must continuously swim in order to obtain adequate oxygen while benthic species have the ability to maintain oxygen levels without swimming (Maia & Wilga, 2013; Wilga & Lauder, 2001). It is also possible that sharks do not respond to barometric pressure changes and, instead, react to other environmental cues during storm passage.

There was a relationship between the change in pressure and the activity per second in the experiments involving *N. crysoleucas*. As the pressure continued to decrease, the distance traveled per second decreased. This conflicts with the common hypothesis that low pressure stimulates a change in activity (Heupel et al., 2003; McCutcheon, 1966). This result could have been because the decrease in manipulated pressure was initiated at a high environmental pressure, cueing the first moment of change and therefore leading to the highest activity. When focusing on the first three hours of the experiment, there was a significant difference between the activity during the periods of decreasing pressure and no change in pressure.

Teleosts and elasmobranchs utilize different sensory adaptations to detect changes in pressure. Teleosts, such as *N. crysoleucas,* possibly utilize their swim bladder (McCutcheon, 1966) while elasmobranchs, such as *C. plagiosum*, could possibly use mechanoreceptors to detect changes in hydrostatic pressure as previously observed with the vestibular hair cells located in the labyrinth of the dogfish sharks (*Scyliorhinus canicula*) (Fraser & Shelmerdine, 2002). The different possible mechanisms to sense pressure may elicit a species-specific response to pressure.

The failure to observe a change in activity in *C. plagiosum* might be explained by the fact that this benthic species may respond by taking refuge as opposed to swimming out of shallow water. It is also possible that barometric pressure change is not the environmental cue that elicits the exodus of sharks from shallow waters.

Auditory Cue of Thunder and Activity

We wanted to test if auditory storm cues could be responsible for changes in activity during storms. We tested whether the auditory cue of "recorded thunder" could trigger increased activity in whitespotted bamboo sharks. The effects of noise in marine organisms have been documented in various studies. One study found that anthropogenic (man-made) noise can alter the behavior of some marine fishes, causing behavior alterations such as startle responses (Peng et al., 2015). Another study found that a tropical storm in Florida increased levels of low-frequency noise. The study found that the nightly chorusing of Black Drum (*Pogonias cromis*) was significantly reduced to less than 10% when compared to pre-storm chorusing during this storm event (Gottesman et al., 2017). The authors concluded that fish communication was negatively impacted by naturally occurring noise during a storm, but they did not consider that the fish may have changed locations during the storm event. If the fish left their natural habitat, it could indicate that there is a response to noise fluctuation during a storm.

A difference in activity of *C. plagiosum* was observed in one thunder experiment but could not be replicated in additional, redesigned, experiments. The redesigned experiments were conducted on different days leading to the possibility of confounding variables having a more significant effect on activity than playing "thunder sounds." One preliminary result suggests that trials conducted on the same day could show a response as there was more activity when the thunder was played than after the thunder was turned off. We were concerned that thunder might alter the subsequent behavior if we played the recorded sounds and then turned it off. Therefore, we decided to conduct trials on different days. The activity of the *C. plagiosum* day to day fluctuates. Therefore, the normal fluctuation in activity in the animals seemed to obscure any effect that may have been present. Since we observed a distinct change in activity when thunder was played the first day, we were surprised that we did not see an apparent effect between days. It is clear that we need a larger sample size and a more controlled environment. While we looked explicitly if the auditory cue of thunder could elicit a response, we did not compare the response to other types of noises.

Responses to a storm have been documented in various marine species, but the majority of those responses were not documented in a controlled environment. The goal of this study was to simulate different environmental cues present within a storm in order to isolate which cues elicit a response. While *C. Plagosium* did not have a significant response to changes in pressure or thunder, more studies need to be conducted with different species of sharks to better understand how sharks respond to storms. Experiments with *N. crysoleucas* did show a relationship between activity and pressure when pressure was decreased. Questions that have yet to be answered include how the rate of pressure change affects animals and the mechanism by which various species detect these changes. The results of this study show that much more research needs to be conducted to understand the complexity of fishes' ability to effectively alter their activity to ensure safety during a storm.

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